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Research Article

Effects of Vitamin E and Selenium Supplementation on Oxidative Stress in Assam Hill and Beetal Crossbred Goats during Transitional Period

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ABSTRACT

Introduction: Oxidative stress during late gestation can adversely affect fetal development, immune function, and overall reproductive performance. The present study aimed to evaluate the effect of antioxidant supplements, including vitamin E and selenium, in reducing oxidative stress (pregnancy stress and heat stress) during the transition period in Assam hill and Beetal crossbred goats.

Materials and methods: A total of 24 healthy pregnant Assam hill and Beetal crossbred goats, each weighing 17-20 kg, were randomly assigned to four groups, each with six goats. The treatment groups consisted of vitamin E at 100 mg and selenium at 0.5 mg (T1), vitamin E at 250 mg and selenium at 1.25 mg (T2), and T3 vitamin E at 500 mg and selenium at 2.5 mg (T3), with these supplements incorporated into their basal diet and administered orally. The control group was given only the basal diet. Different parameters, including hematological parameters (Total erythrocytic count, total leucocytic count, hemoglobin, and packed cell volume), physiological parameters (Temperature, pulse rate, and respiration rate), and biochemical parameters (Total protein, glucose, and cholesterol), were evaluated in four months. Additionally, hormonal levels of Triiodothyronine (T_3), Thyroxine (T_4), and cortisol were assessed, along with oxidative stress biomarkers such as malondialdehyde (MDA) and superoxide dismutase (SOD).

Results: No significant differences were observed among any groups in terms of body temperature, pulse, or respiration rate. The current findings indicated that groups T2 and T3 demonstrated significantly increased serum levels of T3 and T4, as well as an increase in SOD activity, compared to the control group and Group T1. Conversely, groups T2 and T3 indicated a notable reduction in cortisol and MDA concentrations compared to the control group and T1.

Conclusion: Administering 500 mg of vitamin E along with 2.5 mg of selenium has yielded more promising results on oxidative stress; thus, it is recommended for use in pregnant Assam hill and Beetal crossbred goats during the transition period to help reduce stress.

1. Introduction

The livelihood of rural Northeast India predominantly relies on agriculture, livestock husbandry, forestry, and traditional handloom as well as handicraft industries. The majority of rural households engage in small-scale farming and associated activities, with women playing a significant role in both domestic responsibilities and economic pursuits. Due to the mountainous terrain and scattered landholdings, livestock, especially goats, serve as a vital additional income source[1]. When free radicals accumulate in the body, oxidative stress occurs, damaging cellular

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structures and leading to disturbances in normal metabolism and physiological functions[1]. The antioxidative properties of nutrients such as vitamin E and selenium play a crucial role in mitigating pregnancy-related stress by neutralizing excess free radicals produced during the transition period[2]. Oxidative stress during late gestation can negatively impact fetal development, immune function, and overall reproductive performance. Supplementation with antioxidants helps maintain cellular integrity, supports placental function, and improves maternal health outcomes[3]. Vitamin E protects the functional integrity of cellular and subcellular membranes by inhibiting the production of free radicals due to lipid peroxidation[4]. Vitamin E significantly contributes to strengthening the immune system of young small ruminants[5-7], particularly in cases of fetal resumption, testicular degeneration, muscle dystrophy, anemia, and encephalomalacia[8].

Several studies have widely documented that both vitamins and minerals play a significant anti-stress role, positively influencing animal growth, reproductive performance, and overall physiological functions[9]. Vitamin E and selenium provide antioxidant effects through different pathways and safeguard several organs, including the liver, spleen, and lungs, from lipid peroxidation's harmful effects. Selenium, aided by the glutathione peroxidase enzyme, plays a role in detoxifying lipid hydroperoxides and hydrogen peroxide body[10]. Selenium, combined with vitamin E, helps prevent oxygen radicals from oxidizing the membranes of polyunsaturated fatty acids and DNA, thereby protecting them from damage during aerobic processes metabolism[11]. Moreover, the presence of selenium in selenoproteins accounts for its vital role in regulating animal physiology, modulating immune responses, and supporting nervous system function[12].

Therefore, the present study aimed to evaluate the effects of selenium and vitamin E on reducing oxidative stress (pregnancy stress and heat stress) during the transition period in Assam hill and Beetal crossbred goats.

2. Materials and Methods

2.1. Ethical approval

The present experiment was reviewed and approved by the Institutional Animal Ethics Committee (IAEC), AAU, Khanapara, Guwahati, India (Approval no. 770/GO/Re/S/03/CPCSEA/FVSc/AAU/IAEC/21-22/940).

2.2. Study area

The experiment was carried out for four months, from mid-February to mid-June 2022, at the Goat Research Station, Assam Agricultural University, Burnihat, Kamrup, Assam, India. All laboratory analyses were conducted in the Department of Veterinary Physiology, in collaboration with the Department of Veterinary Biochemistry, College of Veterinary Science, Khanapara, Guwahati–781022, Assam, India.

2.3. Experimental design

A total of twenty-four healthy pregnant goats, consisting of four groups with six animals each, were selected for the current study. These goats were crossbreeds of Assam hill and Beetal, with individual body weights ranging from 17 to 20 kg and ages between 16 and 19 months (Figure 1). Goats were maintained at the Goat Research Station, Assam Agricultural University, located in Burnihat, Meghalaya, India. The animals were subjected to experimentation from mid-February to mid-June 2022, during the transition period from advanced pregnancy to early lactation (In goat, 3 weeks before and after kidding). The animals were divided into four groups, each with six goats, including 100 mg vitamin E and 0.5 mg selenium (T1), 250 mg vitamin E and 1.25 mg selenium (T2), and 500 mg vitamin E and 2.5 mg selenium (T3). The control group (C) was provided with the regular basal diet (Table 1). The supplements were administered orally (Figure 2). The goats underwent regular deworming and vaccination in accordance with the guidelines established by the Indian Veterinary Research Institute[13].

Figure 1. Experimental Assam hill and Beetal crossbred goats aged 16 to 19 months

Table 1. Different doses of vitamin E and selenium in experimental groups in Assam hill and Beetal crossbred goats

Group	Treatment
Treatment 1 (T1)	With a regular basal diet and 100 mg Vitamin E and 0.5 mg Selenium
Treatment 2 (T2)	With a regular basal diet and 250 mg Vitamin E and 1.25 mg Selenium
Treatment 3 (T3)	With a regular basal diet and 500 mg Vitamin E and 2.5 mg Selenium
Control (C)	With a regular basal diet and no supplementation

Figure 2. Oral Administration of supplements to different treatment groups in Assam hill and Beetal crossbred goats

All animals in groups T1, T2, T3, and C were systematically monitored for physiological parameters, including temperature, pulse, and respiration rate, employing standard procedures. Blood samples (5 mL) were

collected from the jugular vein in a clot activator, an EDTA vial, and a heparin vial at weekly intervals from the first to the sixth week to evaluate oxidative stress biomarkers such as malondialdehyde (MDA, nmol/g) and superoxide dismutase (SOD, U/ml). Additionally, different hormonal profiles, including triiodothyronine (T_3, nmol/L), thyroxine (T_4, nmol/L), and cortisol (nmol/L), were assessed[14]. The T_3, T_4, and cortisol levels were quantified using radioimmunoassay kits (Beckman Coulter, IMMUNOTECH s.r.o., Czech Republic).

2.4. Basal feed composition and preparation

All animals received commercially prepared feed blocks supplied by the research station, following the standards established by the Indian Council of Agricultural Research, in line with the farm's feeding protocol schedule. Additionally, animals had unlimited access to clean drinking water. The feed blocks were made from locally sourced ingredients, mixed in exact ratios to provide a balanced nutrient profile (Table 2). This mixture was compressed into molds using a hydraulic press and subsequently shade-dried until the blocks solidified, thereby facilitating easy storage and administration. Furthermore, all animals were permitted to graze daily for eight hours on freshly harvested Napier grass (Pennisetum purpureum) cultivated within the research station's premises. The commercially formulated concentrate mixture supplied for the kids was provided by the Indian Council of Agricultural Research (ICAR). For the preparation of feed blocks, the mixture comprised 85% concentrate, 5% molasses, and 10% paddy straw (Figure 3).

Table 2. The ingredients of the feeding block for the Assam hill goats aged 16 to 19 months, according to the Indian Council of Agricultural Research

Ingredients	Percentage (%)
Yellow maize	50
Wheat bran	5
Rice polish	7
Ground nut cake	20
Soyabean	15
Mineral mixture*	2
Common salt	1

* The supplemented Mineral mixture was composed of calcium carbonate, dicalcium phosphate, common salt, magnesium oxide, calcium sulfate, iron sulfate, copper sulfate, zinc sulfate, manganese sulfate, iodized salt, and cobalt sulfate.

Figure 3. Basal feed block for Assam hill and Beetal crossbred goats aged 16 to 19 months

2.5. Sample collection

Blood samples were obtained from each animal on a weekly basis. The samples were collected in EDTA vials for hematological analysis, in heparinized vials for the preparation of haemolysates, and in clot activator vials for serum preparation (Figure 4).

Figure 4. Sample collection in Assam hill and Beetal crossbred goats aged 16 to 19 months

2.6. Statistical analysis

The statistical analysis was carried out according to the standard protocol given by Snedecor and Cochran (1994) [15], by using the software SPSS (Statistical Package for Social Science version 22, Chicago, USA). The analysis of variance (ANOVA) was used to compare the means at 5% level of significance as per Duncan's multiple range test (Duncan, 1995)[16].

3. Results

3.1. Physiological parameters

3.1.1. Temperature

The mean body temperatures of groups C, T1, T2, and T3 were 39.600 ± 0.612, 39.433 ± 0.187, 39.322 ± 0.201, and 39.083 ± 0.220°C in the first week, and 39.367 ± 0.583, 39.233 ± 0.546, 38.733 ± 0.206, and 38.667 ± 0.169°C in the sixth week, respectively. No significant differences were observed among groups, weeks, or their interaction, although a decreasing trend was noted in groups T2 and T3 by the end of the trial ($p > 0.05$).

3.1.2. Pulse rate

The mean pulse rates (per minute) of groups C, T1, T2, and T3 were 80.167 ± 0.946, 79.000 ± 1.065, 78.333 ± 1.453, and 77.833 ± 1.815 in the first week, and 80.167 ± 1.740, 80.667 ± 1.256, 77.667 ± 0.919, and 78.833 ± 1.195 at the sixth week, respectively. No significant differences were observed among groups, weeks, or in the interactions between group and week ($p > 0.05$). However, a gradual decline was noted in groups T2 (77.667 ± 0.919 per minute) and T3 (78.833 ± 1.195 per minute) by the end of the trial.

3.1.3. Respiration rate

The mean respiration rates (per minute) for groups C, T1, T2, and T3 were 17.333 ± 0.989, 15.500 ± 1.057, 18.833

± 1.276, and 17.667 ± 0.667 at the first week, and 16.833 ± 0.749, 17.333 ± 1.116, 19.667 ± 0.422, and 19.000 ± 1.065 in the sixth week, respectively. No significant differences were found among groups, weeks, or in the interactions between group and week ($p > 0.05$). However, a slight decreasing trend in respiration rate was observed in groups T2 (19.667 ± 0.422 per minute) and T3 (19.000 ± 1.065 per minute) with higher antioxidant doses toward the end of the trial.

3.2. Hormonal profiles

3.2.1. Triiodothyronine

The T_3 levels in groups C, T1, T2, and T3 were 2.203 ± 0.098, 2.396 ± 0.177, 2.269 ± 0.103, and 2.305 ± 0.093 (nmol/L) in the first week, and 1.963 ± 0.099, 2.005 ± 0.120, 2.361 ± 0.103, and 2.574 ± 0.077 (nmol/L) in the sixth week, respectively. The present study demonstrated statistically significant differences among the groups and across different weeks ($p < 0.05$). A statistically significant interaction between groups and weeks was observed ($p < 0.05$), with notable changes in Group T2 beginning from the third week and in Group T3 starting from the second week onward (Table 3, Figure 5).

Table 3. Level of triiodothyronine, thyroxine, and cortisol in pregnant Assam hill and Beetal crossbred goats aged 16 to 19 months

Parameter	Control			Treatments								
				T1			T2			T3		
Week	Triiodothyronine	Thyroxine	Cortisol	Triiodothyronine	Thyroxine	Cortisol	Triiodothyronine	Thyroxine	Cortisol	Triiodothyronine	Thyroxine	Cortisol
First	2.203 ± 0.098^{a}	47.975± 2.563^{a}	52.113 ± 4.179^{a}	2.396 ± 0.177^{a}	51.420± 2.720^{a}	52.815 ± 2.781^{a}	2.269 ± 0.103^{ab}	53.770± 2.581^{ab}	49.110± 2.347^{a}	2.305 ± 0.093^{a}	48.458 ± 2.705^{a}	53.167 ± 1.950^{a}
Second	1.882 ± 0.155^{a}	46.292± 2.262^{a}	49.802 ± 2.352^{a}	2.063 ± 0.137^{a}	49.382± 1.382^{a}	51.183 ± 1.359^{a}	2.182 ± 0.073^{ab}	48.285± 1.602^{a}	42.065± 1.971^{b}	2.417 ± 0.047^{ab}	46.232 ± 3.455^{a}	41.942 ± 2.200^{b}
Third	1.718 ± 0.121^{a}	42.507± 2.225^{a}	51.962 ± 1.926^{a}	2.026 ± 0.105^{b}	42.617± 3.110^{a}	50.642 ± 2.026^{a}	2.132 ± 0.09^{a}	46.568± 1.684^{a}	43.03 ± 2.611^{b}	2.520 ± 0.043^{b}	47.390 ± 3.842^{a}	36.305 ± 1.421^{c}
Fourth	1.606 ± 0.100^{a}	38.245± 3.699^{a}	54.322 ± 3.891^{a}	1.884 ± 0.080^{b}	40.203± 3.096^{a}	50.123 ± 0.861^{ab}	2.127 ± 0.118^{a}	44.065± 3.737^{ab}	41.487± 2.963^{bc}	2.484 ± 0.049^{ab}	50.968 ± 3.197^{b}	32.542 ± 5.338^{c}
Fifth	1.580 ± 0.155^{a}	41.253± 3.163^{a}	49.918 ± 2.845^{a}	1.843 ± 0.141^{ab}	42.568± 1.053^{a}	47.410 ± 1.491^{a}	2.133 ± 0.111^{a}	48.963± 3.242^{ab}	39.080± 2.121^{bc}	2.534 ± 0.089^{b}	56.257 ± 2.038^{b}	30.477 ± 2.125^{c}
Sixth	1.963 ± 0.099^{a}	46.265± 1.148^{a}	46.945 ± 5.751^{a}	2.005 ± 0.120^{ab}	49.625± 1.877^{a}	43.792 ± 1.560^{a}	2.361 ± 0.103^{b}	56.085± 2.908^{b}	38.378± 1.922^{c}	2.574 ± 0.077^{b}	63.565 ± 2.269^{c}	30.743 ± 2.758^{c}

Values are presented as mean ± standard deviation. $^{a, b, c, \text{and } d}$ Different superscript letters in the same column differ significantly among groups ($p < 0.05$).

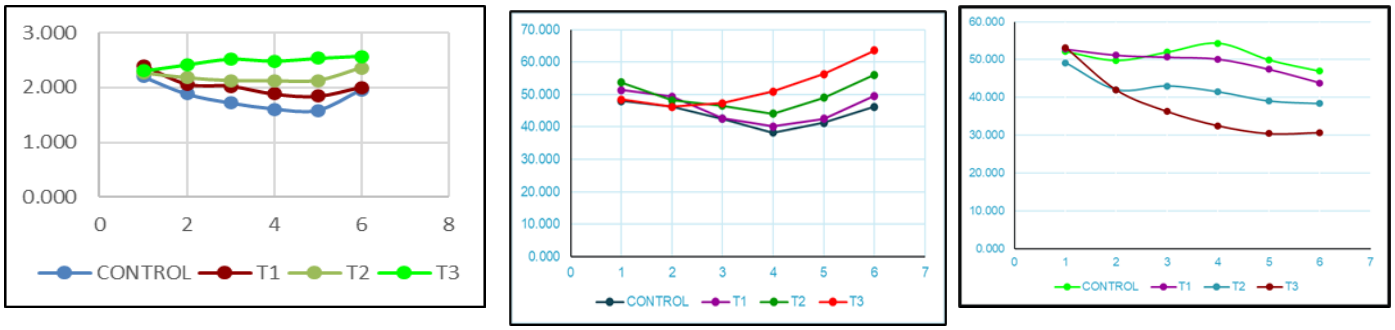

Figure 5. Mean triiodothyronine, thyroxine, and cortisol values of different experimental groups at weekly intervals

3.2.2. Thyroxine

The level of T_4 in groups C, T1, T2, and T3 was found to be 47.975 ± 2.563, 51.420 ± 2.720, 53.770 ± 2.581, and 48.458 ± 2.705 (nmol/L), and 46.265 ± 1.148, 49.625 ± 1.877, 56.085 ± 2.908, and 63.565 ± 2.269 (nmol/L), respectively, at the first and sixth week. The mean T_4 value exhibited a statistically significant difference among the groups and weeks ($p < 0.05$). However, interaction among groups T2 and T3 and weeks was found to be statistically significant ($p < 0.05$). Among all the groups, T3 indicated the highest T_4 value (63.565 ± 2.269 nmol/L) in the sixth week (Table 3, Figure 5).

3.2.3. Cortisol

The cortisol level in groups C, T1, T2, and T3 at first and sixth weeks was found to be 52.113 ± 4.179, 52.815 ± 2.781, 49.110 ± 2.347, and 53.167 ± 1.950 (nmol/L), and 46.945 ± 5.751, 46.945 ± 5.751, 38.378 ± 1.922, and 30.743 ± 2.758 (nmol/L), respectively. The mean cortisol value was found to significantly differ among the groups and between weeks ($p < 0.05$). The interaction across groups and weeks indicated a significant difference ($p < 0.05$). The level of cortisol indicated a significant decrease in groups T2 and T3 from the second week onwards. highest levels of cortisol compared to the treatment groups (52.113 ± 4.179 nmol/L, Table 3, Figure 5).

3.3. Oxidative stress biomarker

3.3.1 Malondialdehyde

The MDA levels in groups C, T1, T2 and T3 at first and sixth week were found as 5.927 ± 0.107, 5.523 ± 0.192, 5.710 ± 0.179, and 5.678 ± 0.190 (nmol/g), and 5.055 ± 0.082, 4.505 ± 0.130, 4.390 ± 0.157, and 3.867 ± 0.144 (nmol/g), respectively (Table 4, Figure 6). The present study revealed that in all treatment groups, the concentration of MDA was found to decrease across the weeks, from the first week to the sixth week ($p < 0.05$). The MDA concentration in Group T2 was higher than in groups T1 and T3. In the control group, the MDA values exhibited a similar trend from the first to the sixth week of pregnancy. The mean MDA values of the animals indicated a significant difference among the groups and across weeks ($p < 0.05$). However, the interaction between group and weeks differed significantly ($p < 0.05$).

Table 4. Level of malondialdehyde and superoxide dismutase in pregnant Assam hill and Beetal crossbred goats, and body weight gain in kids of experimental groups

Parameter	Control		Treatment					
			T1		T2		T3	
Week	Malondialdehyde (nmol/g of Hb)	Superoxide dismutase	Malondialdehyde (nmol/g of Hb)	Superoxide dismutase	Malondialdehyde (nmol/g of Hb)	Superoxide dismutase	Malondialdehyde. (nmol/g of Hb)	Superoxide dismutase
First	$5.927^{a} \pm 0.107$	$1.782^{a} \pm 0.003$	$5.523^{a} \pm 0.192$	$1.780^{a} \pm 0.004$	$5.710^{b} \pm 0.179$	$1.777^{a} \pm 0.005$	$5.678^{a} \pm 0.190$	$1.785^{a} \pm 0.004$
Second	$5.908^{a} \pm 0.085$	$1.770^{a} \pm 0.004$	$5.493^{ab} \pm 0.160$	$1.772^{a} \pm 0.004$	$5.382^{b} \pm 0.098$	$1.775^{a} \pm 0.004$	$5.200^{a} \pm 0.199$	$1.788^{a} \pm 0.005$
Third	$5.717^{a} \pm 0.180$	$1.783^{a} \pm 0.007$	$5.297^{ab} \pm 0.160$	$1.783^{a} \pm 0.005$	$5.025^{b} \pm 0.099$	$1.797^{ab} \pm 0.006$	$4.697^{b} \pm 0.180$	$1.812^{b} \pm 0.006$
Fourth	$5.603^{a} \pm 0.152$	$1.788^{ab} \pm 0.006$	$5.198^{b} \pm 0.184$	$1.775^{a} \pm 0.004$	$4.985^{b} \pm 0.019$	$1.803^{ab} \pm 0.005$	$4.422^{b} \pm 0.082$	$1.820^{c} \pm 0.007$
Fifth	$5.435^{a} \pm 0.168$	$1.780^{a} \pm 0.004$	$5.005^{b} \pm 0.153$	$1.778^{a} \pm 0.005$	$4.770\ a^{b} \pm 0.130$	$1.800^{b} \pm 0.004$	$4.063^{b} \pm 0.034$	$1.817^{c} \pm 0.006$
Sixth	$5.055^{a} \pm 0.082$	$1.777^{a} \pm 0.005$	$4.505^{c} \pm 0.130$	$1.775^{a} \pm 0.004$	$4.390^{a} \pm 0.157$	$1.792^{a} \pm 0.005$	$3.867^{c} \pm 0.144$	$1.812^{c} \pm 0.006$

Values are presented as mean ± standard deviation. [a, b, c, and d] Different superscript letters in the same column differ significantly among groups ($p < 0.05$).

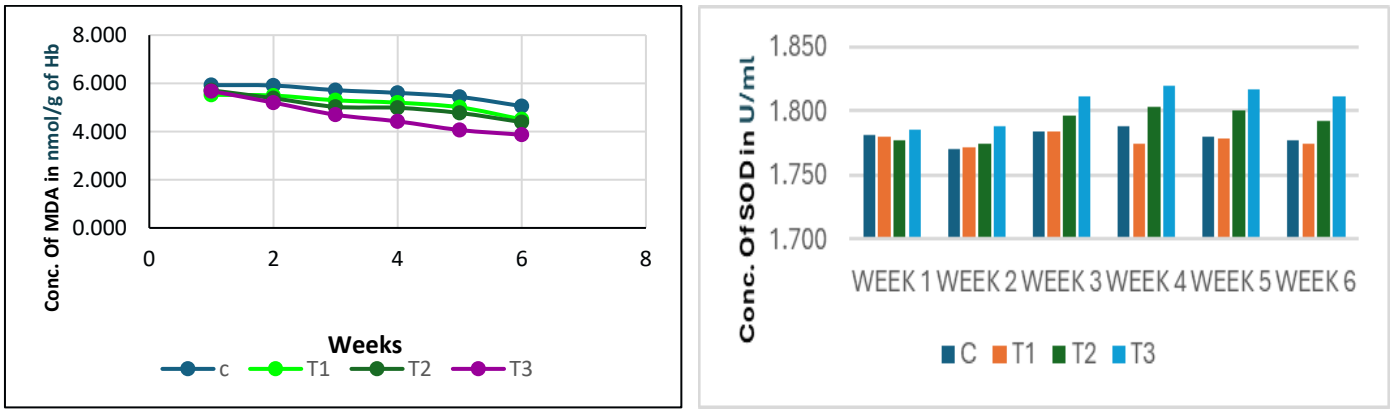

Figure 6. Mean malondialdehyde and superoxide dismutase values of different experimental groups at weekly intervals

3.3.2. Superoxide dismutase

The SOD level (Mean ± SD) in groups C, T1, T2, and T3 during the first and sixth weeks was recorded as 1.782 ± 0.003, 1.780 ± 0.004, 1.777 ± 0.005, and 1.785 ± 0.004 (U/ml), respectively, with values of 1.777 ± 0.005, 1.775 ± 0.004, 1.792 ± 0.005, and 1.812 ± 0.006 (U/ml) observed in the sixth week. The present study revealed that SOD (Mean ± SD) varied among groups T2 and T3, showing increases during the fourth and fifth weeks of treatment. The ANOVA indicated statistically significant differences ($p < 0.05$) among the groups and also showed significant weekly variation ($p < 0.05$; Table 4, Figure 6). The mean superoxide dismutase activity of the animals displayed significant ($p < 0.05$) differences both between groups and across weeks. Significant differences were observed in the interaction between groups and weeks. Group T3 showed the highest value among all the groups.

4. Discussion

4.1. Physiological parameters

The current findings align with those of Shakirullah et al.[17], who highlighted the importance of physiological parameters, such as pulse rate, rectal temperature, and respiration rate, as dependable indicators of stress during pregnancy. Activation of the hypothalamic-pituitary-adrenal axis in response to pregnancy-related stress causes elevated cortisol levels, often leading to an increased pulse rate18. In the present study, the decrease in pulse rate seen in the antioxidant-supplemented groups may be due to the combined effects of vitamin E and selenium, which are known to reduce oxidative stress and promote homeostatic functions[19].

Stress during pregnancy, particularly when combined with handling or environmental challenges, can adversely impact maternal health and fetal development. Antioxidant supplementation appears to enhance the resilience of animals to these stresses by reducing cellular oxidative damage. This

likely facilitated the stabilization of pulse and respiratory rates. The present findings are consistent with those of Shakirullah et al.[20], who reported improved physiological responses in pregnant animals administered vitamin E and selenium supplements.

4.2. Hormonal profiles

The thyroid gland is the most vulnerable organ to thermal gradient stress, according to Shakirullah et al.[20]. Thyroid hormones are known to play a crucial role in regulating overall metabolism and developmental processes, as stated by Rasooli et al.[21]. The current findings may be linked to a selenium-dependent enzyme called type I iodothyronine-5´-deiodinase, which helps convert T_4 to T_3 through deiodination. Alternatively, selenium might be necessary for the liver to convert T_4 into 3,3',5-triiodothyronine. Type I iodothyronine deiodinase is a selenocysteine-containing enzyme that catalyzes the deiodination of T4 to produce biologically active thyroid hormone22.

According to Solimon23, vitamin E and selenium injection significantly increased T_3 levels in lambs, which is consistent with the present results. Sethy et al.[24] reported an increase in T_3 after supplementing their diet with selenium. The present study revealed similar results to those of Sivakumar et al.[25]. Shinde et al.[26] conducted a 180-day experiment on buffaloes, supplementing them with 0.3 ppm of selenium and 300 IU of DL-alpha-tocopherol acetate, resulting in similar outcomes for T3 hormone. Conversely, Kumar et al.[27] found no impact of selenium supplementation on lambs, regardless of whether organic or inorganic forms of T_3 were used. The variation in findings may be attributable to the dose rate of 0.15 ppm. It is conceivable that this lower dose was inadequate to fulfill the physiological requirements for optimal enzymatic activity involved in thyroid hormone metabolism, particularly the activity of selenium-dependent deiodinase enzymes responsible for converting T_4 to the more active T_3 form. The threshold level of selenium necessary to activate these enzymes effectively may differ depending on species, age, physiological condition, and baseline selenium status of the subjects. Consequently, the suboptimal dose of 0.15 ppm employed in the study of Kumar et al.27 might not have been sufficient to produce a measurable increase in T_3 levels, in contrast to the higher dose (0.3 ppm) applied in the current and other supporting investigations.

The present findings were similar to those of Sivakumar et al.[25] and Shakirullah et al.[20], who reported that vitamin E and selenium supplementation significantly increased serum T_4 levels. This increase can be attributed to selenium's role as a cofactor in iodothyronine deiodinases, which convert T_4 to the biologically active T_3[28]. Vitamin E supports thyroid function by reducing oxidative damage to the gland[15]. The elevated T_3 and T_4 levels observed in the present study suggested improved thyroid activity and metabolic adaptation, especially important during pregnancy.

However, unlike these findings, Ramadan et al.[29] found no significant changes in thyroid hormones or plasma metabolites. This difference might be due to variations in dosage, the animals' physiological condition, or the duration of supplementation, indicating that achieving an effective response could require optimal levels of selenium and vitamin E.

Comparable findings were also documented by Dimri et al.[30] concerning T_4 levels, in experiments on 42 healthy, lactating water buffaloes. The results demonstrated significant alterations at 15 and 45 days postpartum. Gupta et al.[31] examined the impact of vitamin E and selenium on plasma cortisol levels in pregnant cows and observed a notable decrease in plasma cortisol concentration from 21 days prior to parturition until the day of calving in cows supplemented with vitamin E and selenium. During periods of stress, the body generates free radicals. Cortisol serves as a reliable stress marker in cattle and other animals; consequently, its production increases during stress[32]. In the current study, the antioxidant properties of selenium and vitamin E mitigate the number of free radicals within the body, thereby facilitating a reduction in cortisol levels and alleviating oxidative stress[32].

4.3. Oxidative stress biomarker

4.3.1. Malondialdehyde

The MDA is produced as a result of lipid peroxidation, which occurs after oxidative degradation of lipids by reactive oxygen species (ROS)[19]. This lipid peroxidation process results in the degradation of cell membrane lipids, which damages cells and ultimately puts the animal under oxidative stress. According to Nielsen and Menné[33], MDA can be stated as a marker of the oxidative damage caused by ROS and lipid peroxidation.

The mean MDA values of the animals demonstrated a statistically significant difference among groups T1, T2, and T3. Although a significant difference was observed, the MDA concentration seemed to decline over the weeks. Vitamin E and selenium are well recognized as antioxidants possessing anti-peroxidative properties[34]. The synergistic mechanism of selenium and vitamin E is expressed as the body's main antioxidants, helping to neutralize lipid peroxyl radicals[35]. Thus, it has been hypothesized that vitamin E and selenium administration may have reduced MDA production in the liver by protecting it from lipid peroxidation and cell membrane damage[36]. The significantly lower MDA levels seen in vitamin E and selenium-treated animals aligned with the findings reported by Khatti et al.[36] and Mahmood et al.[37].

4.3.2 Superoxide dismutase

During a healthy pregnancy, the antioxidative defense system adapts, with studies showing a decrease in SOD activity in mammals[38]. The primary antioxidant enzymes in mammalian cells are catalase and SOD. Additionally, both catalase and glutathione peroxidase work together with SOD as enzymes that eliminate H2O2[31]. Lipid peroxides, formed as reactive oxidized by-products of lipids, can damage cells and disrupt cell membranes[38,39]. Consequently, oxidative stress occurs and is linked to the development of

several disorders in ruminants during the periparturient stage[40], such as reproductive disorders[41,42] and mastitis[43,44].

The present findings agree with those of Khatti et al.[36], who reported an effect of treatment on serum SOD activity. Similarly, Dhanasree et al.[45] observed comparable results in their experiment on caprine. Sahin et al.[35] obtained similar findings on Japanese quail regarding the protective role of vitamin E and selenium. The current results align with those of Mahmood et al.[37], who reported similar outcomes in their experiment involving 36 multiparous pregnant and non-pregnant goats. The treatment group received vitamin E (1000 mg/kg body weight) and selenium (3 mg/50 kg body weight). Plasma SOD levels in pregnant goats were not marginally elevated but decreased abruptly after delivery. Moreover, the treatments significantly affected plasma SOD activity during the expected parturition period (150 days).

5. Conclusion

It can be concluded that dietary supplementation with 500 mg of vitamin E and 2.5 mg of selenium demonstrated greater efficacy in mitigating stress during pregnancy and the transition period in Assam hill and Beetal crossbred goats. Further studies are needed at different levels to evaluate the effects of vitamin E and selenium on different blood parameters in goats.

Declarations

Ethical consideration

The author has reviewed all ethical problems, including plagiarism, consent to publish, data fabrication, and falsification, before publishing.

Competing interests

The authors declared no competing interests.

Authors' contributions

The corresponding author, Salima Siddika, was principally responsible for carrying out the laboratory testing, writing the report, and conducting the experimental studies. As the principal mentor, Champak Barman offered crucial advice and guidance during the study. Arundhati Bora supported the laboratory analysis in the Department of Physiology and provided crucial support as a co-guide. As supporting guides, Devojyoti Dutta and Prithviraj Mazinder Barua offered broad guidance throughout the experimental study. Iqbal Salik Minhaz supported the study by providing technical assistance during the laboratory experiments and guidance during data analysis. The final edition of the manuscript was read and approved by all authors.

Availability of data and materials

The data is available with the author and will be provided upon a fair request.

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